

Exploring edible bird nest's potential in mitigating Wi-Fi's impact on male reproductive health

Sofwatul Mokhtarah Maluin¹  | Farah Hanan Fathihah Jaffar² | Khairul Osman³ | Aini Farzana Zulkefli² | Mohd Farisyam Mat Ros² | Siti Fatimah Ibrahim²

¹Department of Physiology, Faculty of Medicine and Health Sciences, Universiti Sains Islam Malaysia (USIM), Nilai, Malaysia

²Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM), Kuala Lumpur, Malaysia

³Centre of Diagnostic Science and Applied Health, Faculty of Health Sciences, Universiti Kebangsaan Malaysia (UKM), Bangi, Malaysia

Correspondence

Siti Fatimah Ibrahim, Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM), Kuala Lumpur, Malaysia.
Email: timi@ukm.edu.my

Funding information

Universiti Kebangsaan Malaysia, Grant/Award Number: FF-2021-200

Abstract

Purpose: This study aimed to evaluate the protective effects of edible bird nest (EBN) against the detrimental impact of Wi-Fi on male reproductive health. Specifically, it examines whether EBN can mitigate Wi-Fi-induced changes in male reproductive hormones, estrogen receptors (ER), spermatogenesis, and sperm parameters.

Methods: Thirty-six adult male rats were divided into six groups ($n=6$): Control, Control EBN, Control E2, Wi-Fi, Wi-Fi+EBN, and Wi-Fi+E2. Control EBN and Wi-Fi+EBN groups received 250mg/kg/day EBN, while Control E2 and Wi-Fi+E2 groups received 12µg/kg/day E2 for 10days. Wi-Fi exposure and EBN supplementation lasted eight weeks. Assessments included organ weight, hormone levels (FSH, LH, testosterone, and E2), ERα/ERβ mRNA and protein expression, spermatogenic markers (c-KIT and SCF), and sperm quality.

Results: Wi-Fi exposure led to decreased FSH, testosterone, ERα mRNA, and sperm quality (concentration, motility, and viability). EBN supplementation restored serum FSH and testosterone levels, increased serum LH levels, and the testosterone/E2 ratio, and normalized mRNA ERα expression. Additionally, EBN increased sperm concentration in Wi-Fi-exposed rats without affecting motility or viability.

Conclusions: EBN plays a crucial role in regulating male reproductive hormones and spermatogenesis, leading to improved sperm concentration. This could notably benefit men experiencing oligospermia due to excessive Wi-Fi exposure.

KEYWORDS

edible bird nest, infertility, radiofrequency radiation, sperm, Wi-Fi

1 | INTRODUCTION

Edible bird nest (EBN), known for its multifaceted properties such as hormonal, antioxidant, and cellular proliferative properties, has emerged as a prospective route for investigation in the context of male reproductive health.^{1,2} Amid a global surge in male infertility

issues, the potential of EBN as a potential mitigator of the adverse effects of Wi-Fi radiation has gained interest from researchers and the public.^{3,4}

Wi-Fi usage has grown inevitably linked to economic growth and social advancement as a fundamental component of modern living. On the other hand, the radiofrequency electromagnetic field

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial-NoDerivs](https://creativecommons.org/licenses/by-nc-nd/4.0/) License, which permits use and distribution in any medium, provided the original work is properly cited, the use is non-commercial and no modifications or adaptations are made.

© 2024 The Author(s). *Reproductive Medicine and Biology* published by John Wiley & Sons Australia, Ltd on behalf of Japan Society for Reproductive Medicine.

(RF-EMF) radiation emitted by Wi-Fi devices' safety has raised serious concerns among medical professionals. The radiation has been reported to produce thermal and nonthermal effects on humans. These effects are primarily concentrated on the testicular micro-environments. Chronic exposure would consequently impair spermatogenesis and sperm quality.⁵

The intricate regulation of spermatogenesis relies on male reproductive hormones, including gonadotropin-releasing hormone (GnRH), follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and estrogen. These hormones operate in a coordinated manner to maintain healthy sperm production. However, studies have shown that the delicate balance of this process can be disrupted by RF-EMF radiation. This disruption is associated with decreased testosterone levels, impaired proliferation of spermatogonia, increased damage to sperm DNA, and a decline in sperm quality.^{6,7}

A crucial aspect warranting further investigation is the impact of Wi-Fi on estrogen and its influence on male reproduction, which remains undiscovered as of now. Estrogen plays a critical role and is produced in Leydig adult and testicular germ cells through the conversion of testosterone by cytochrome P450 aromatase.⁸ Its effects are mediated through estrogen receptors (ER), specifically ER α and ER β , which are present in various testicular cells and play a pivotal role in different stages of spermatogenesis.⁹ A reduction in estrogenic activity is associated with lower expression of ER and has been linked to impaired spermatogenesis.¹⁰ Despite recognizing the critical role of estrogen in male reproduction, the specific effects of Wi-Fi radiation exposure on estrogen levels and its subsequent consequences for male reproductive health remain unclear. This underscores a notable gap in our understanding of this pathway.

The Wi-Fi exposure model was chosen for this study due to its relevance to common environmental RF-EMF exposure, which is crucial for examining its impact on male reproductive health. Existing research demonstrates that RF-EMF from Wi-Fi can adversely affect sperm quality and reproductive hormones, making it a suitable model for assessing the potential benefits of EBN. This model simulates consistent, chronic exposure scenarios, reflecting real-life conditions and ensuring that the research findings are applicable to everyday situations.

Therefore, in this study, we examined the impact of Wi-Fi exposure on male reproductive hormones, encompassing FSH, LH, testosterone, and estrogen. Additionally, we explored its influence on testicular estrogenic activity and spermatogenesis by assessing c-KIT and SCF as markers for spermatogenic proliferation. Ultimately, we aimed to elucidate how these factors collectively contributed to sperm quality. Furthermore, the objective of this study was to evaluate the potential advantages of incorporating EBN as a supplement to alleviate the impact of Wi-Fi exposure on all these parameters.

EBN shows promise as a supplement for male reproductive health, exhibiting properties that encompass reproductive hormones such as testosterone, estrogen, LH, FSH, and prolactin.^{2,11}

It has also been proven to possess antioxidant and cell proliferation properties.^{1,12} In addition, EBN has been shown to have estrogenic attributes, exhibiting favorable outcomes in female reproduction. However, more research is needed to determine the effect of EBN on male estrogen levels and spermatogenesis. It is critical to investigate EBN's estrogenic activities in relation to male reproduction and its impact on sperm quality. A recent study by Jaffar et al.¹¹ found that EBN supplementation increased sperm counts, highlighting the potential advantages for male reproductive health.

The outcomes of this study will provide valuable insights into how Wi-Fi radiation impacts male reproductive health, the potential protective role of EBN, and its suitability as a supplement to address male infertility.

2 | MATERIALS AND METHODS

2.1 | Animals

Thirty-six male Sprague–Dawley rats (8 weeks), with an average weight of 200–250 g, were obtained from the Laboratory Animal Resources Unit of the Faculty of Medicine, Universiti Kebangsaan Malaysia (UKM). The rats were housed in a standard environment prior to the experiment to simulate real-world conditions where Wi-Fi exposure is ubiquitous. During the experiment, the rats were individually housed in cages at a temperature of $22 \pm 3^\circ\text{C}$, maintaining a 12:12 h light–dark cycle in the animal house at Fakulti Sains dan Teknologi, UKM, Bangi. Mouse pellets and water were given ad libitum to the rats. Nonautoclaved wood shavings were used as bedding and changed weekly to maintain a clean environment for the rats. This animal study was approved by the Animal Ethics Committee UKM (UKMAEC) with approval number FISIO/FP/2021/SITI FATIMAH/24-MAR./1155-MAR.-2021-JULY-2022.

2.2 | Experimental design

Rats were randomly divided into six groups: Control, Control EBN, Control E2, Wi-Fi, Wi-Fi+E2, and Wi-Fi+EBN (Table 1). Before the experiment commenced, blood samples were collected from all rats in each group for baseline hormonal analysis. Subsequently, the rats were subjected to eight weeks of Wi-Fi exposure and EBN supplementation.

2.3 | Wi-Fi exposure setting

For Wi-Fi exposure, this study utilized the TP-LINK AC750 Wireless Dual Band Wi-Fi Router Archer C20 (Shenzhen, China). This router features three external antennas, emitting signals at a frequency of 2.45 GHz using the IEEE 802.11n standard. The router was positioned 20 cm away from the rat cages and constantly exchanged data with a Raspberry Pi device through a ping

TABLE 1 Animal grouping, type of feed, and Wi-Fi exposure status.

Group	Group assign	Type of feed (dose)/with or without exposure to Wi-Fi
Control	C	SD/without Wi-Fi exposure
Control EBN	CEBN	SD+EBN (250 mg/kg)/without Wi-Fi exposure
Control E2	CE	SD+E2 (12 µg/kg)/without Wi-Fi exposure
Wi-Fi	W	SD/2.45 GHz Wi-Fi exposure
Wi-Fi+E2	WE	SD+E2 (12 µg/kg)/2.45 GHz Wi-Fi exposure
Wi-Fi+EBN	WEBN	SD+EBN (250 mg/kg)/2.45 GHz Wi-Fi exposure

Abbreviations: E2, 17 β -estradiol; EBN, edible bird nest; SD, standard diet.

protocol.³ The router was selected for its minimal vibration and noise output, which is typical for standard Wi-Fi routers used in laboratory settings. Additionally, the rats were housed in a controlled environment designed to minimize external disturbances, ensuring the reliability of our findings related to Wi-Fi exposure effects on male reproductive health.

2.4 | Edible bird's nest supplementation

The unprocessed EBN was sourced from a specific swiftlet's habitat in Bera, Pahang, Peninsular Malaysia. After collection, the EBN underwent processing and freeze-drying by Glycofood Sdn Bhd, Malaysia. The dosage and supplementation approach selection followed our prior publication.¹¹ Jaffar et al.⁶ previously conducted a profile analysis of EBN, revealing the presence of reproductive hormones such as testosterone, estradiol, and progesterone. Additionally, sialic acid, amino acids, vitamin K, calcium, and magnesium iodide were also identified in the composition.

2.5 | Estradiol (E2) administration

Estradiol (E2) stock solution was made by dissolving 10 µg of E2 in 0.1 mL of peanut oil. It was then diluted with peanut oil to create a final 20 µg/mL concentration. E2 and Wi-Fi+E2 rats received E2 subcutaneous injections (12 µg/kg) for 10 days. This dosage was chosen based on Olfati et al.,¹³ which showed E2's positive effects on sperm quality after busulfan-induced impairment. As EBN has estrogenic properties, E2 treatment groups were included to compare to the various EBN supplementation groups. This comparison assessed whether EBN's estrogenic effects resemble externally administered E2.

2.6 | Animal euthanization, blood and tissue sampling

After eight weeks of Wi-Fi exposure and EBN treatment, rats were euthanized. An anesthesia overdose (ketamine/xylazine/zoletil—KTX) was administered through intraperitoneal injection before dissection. Once the rats were sacrificed, blood was collected using cardiac puncture and allowed to clot for two hours at room temperature. The

serum was then separated by centrifugation at 1000g for 15 min at 2–8°C and stored in –80°C for subsequent ELISA analysis.

The testes, epididymis, and seminal vesicles on both sides were carefully dissected, separated from surrounding adipose tissue, and weighed. Organ weights were represented as organ coefficients. It was calculated by dividing individual rat weights by the respective organ weights.¹⁴ The epididymis was then immersed in warm PBS for sperm parameter evaluation. At the same time, the excised testes were promptly frozen in liquid nitrogen and stored at –80°C for later protein and RNA extraction.

2.7 | Sperm analysis

Sperm collection from cauda epididymis involved mincing the tissue and placing it in a prewarmed PBS solution (2 mL). The mixture was then gently pipetted up and down using a P-1000 pipette with large orifice tips to release the sperm. After a 30-min incubation at 37°C, sperm parameters were measured. A 10 µL sperm suspension was placed on a Makler Chamber, and the number of sperm in 10 grids was counted for average sperm concentration.

Sperm motility was assessed based on the WHO 2010 classification, which includes progressive motility (PR), nonprogressive motility (NP), and immotility (IM). A 10 µL sperm suspension drop was evaluated under a microscope at 40× magnification, and PR, NP, and IM sperm were counted in duplicate to determine the percentage of motile sperm.

Sperm viability was determined using the hypo-osmotic swelling test (HOST). Sperm were mixed in a 1:10 ratio with a hypo-osmotic solution and incubated. A stained smear was examined under a microscope at 40× magnification to count the viable (swollen) and nonviable (unswollen) sperm. The percentage of viable sperm was calculated based on these counts.

2.8 | Serum follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol determination by ELISA

Serum levels of follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, and estradiol (E2) were measured using enzyme-linked immunosorbent assay (ELISA) kits. The ELISA

Gene symbol	Primer sequence (5'-3')	NCBI gene ID
ESR1	Forward: ATATGATCAACTGGGCAAAG Reverse: CATTACCTTGATTCTCCTGTCC	NM_012689 XM_039101049 XM_017588797 XM_039101051
ESR2	Forward: GGAAATCTTTGACATGCTCC Reverse: GGTACATACTGGAGTTGAGG	XM_006240221 NM_012754
Kit (c-KIT)	Forward: ATTTGGAAAAGTGGTGGTC Reverse: GATTGCTCTTTGCTGTTAC	XM_006250909.4 NM_022264.2
Kitlg (SCF)	Forward: GTGCTCTCTTCAACATTAGG Reverse: CTTGACTGTTTCTTCTTCCAG	NM_021843.4 NM_021844.2
Beta-actin	Forward: AAGACCTCTATGCCAACAC Reverse: TGATCTTCATGGTGTAGG	NM_031144 XM_039089807
Cyclin A2	Forward: AGGGAAATGGAGGTAAATG Reverse: CTATCAATGTAGTTCACAGCC	NM_053702 XM_008760903

TABLE 2 Primer sequences and gene numbers for estrogen receptor, c-kit, SCF, and housekeeping genes.

process followed the recommended manufacturer's instructions (Elabscience, Wuhan, China).

A Sandwich-ELISA approach was used for FSH and LH. Standard solutions and serum samples were added to precoated plates in duplicate. After incubation and washing, biotinylated detection antibodies were added, followed by an Avidin-HRP Conjugate solution. The solution optical density (OD) was measured at 450nm, proportional to FSH/LH concentration.

A competitive ELISA method was used for testosterone and E2. Standard and serum samples were added to plates, followed by a Biotinylated Detection Antibody solution. After incubation and washing, the Avidin-HRP conjugate solution was added. The OD was measured at 450nm, which is inversely related to the concentration of testosterone/E2. The concentration was determined by comparing the OD to a standard curve generated from known concentrations of testosterone and E2 provided by manufacturer.

2.9 | Evaluation of estrogen receptors, c-KIT and stem cell factor protein expression by Western blot

Small rat testes segments (0.3g) were washed with cold PBS and homogenized on ice in RIPA lysis buffer with a protease inhibitor (Elabscience, Wuhan). The supernatants were collected after centrifugation at 12000rpm for 10min at 4°C. Protein concentration was determined using the Bradford Assay and measured at 595nm.

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was conducted with 10% separating gel (ER and SCF) or 8% separating gel (c-KIT) and 4% stacking gel. Protein samples (20µg) were mixed with 5× SDS loading buffer, heated at 70°C for 5min, and centrifuged. Sample proteins (10µL/well) were then loaded onto the gel, and electrophoresis was run at 150V for 45min. Then, proteins were transferred to PVDF membranes at 40V for two hours at 4°C. After blocking with 5% skim milk, membranes were incubated overnight at 4°C with primary antibodies anti-mouse ERα, ERβ, c-KIT, or SCF (Santa Cruz, Delaware Avenue, CA, USA).

Following washing, the secondary antibody HRP-conjugated goat anti-mouse IgG (Elabscience, Wuhan, China) was added and incubated for an hour. Chemiluminescent substrate (ECL, Elabscience, Wuhan, China) was used for detection, and the membrane was read using Gel Doc Amersham Imager 600. For B-actin detection, the PVDF membrane was stripped using a stripping buffer (Elabscience, Wuhan, China). After blocking and incubation with an anti-mouse B-actin antibody, a secondary antibody was added, followed by protein detection and visualization. The band densities for each group were normalized to B-actin in the same group using ImageJ version 1.52a.

2.10 | qPCR analysis of estrogen receptors, c-KIT and stem cell factor in the testis

Total RNA was extracted from rat testes using an RNA extraction kit (Vivantis, Malaysia). About 30mg of testicular tissue was ground into a fine powder with liquid nitrogen, homogenized, and then centrifuged to collect the lysate. Ethanol was added for RNA isolation using an RNA-binding column. After washing, DNase treatment was applied for 15min at room temperature. Subsequently, inhibitor removal solution was added, followed by two rounds of column washing. The eluted RNA was collected in a new tube with RNase-free water and centrifuged.

A nanodrop machine was used to determine RNA concentration in ng/µl and RNA purity using A260/280. The A260/280 values for all extracted RNA samples were within the manufacturer's recommended range of 2.0–2.1. For cDNA synthesis, an RNA-primer mixture was prepared using total RNA, oligo d(T)18, random hexamers, and dNTPs. After incubation and cooling, the cDNA synthesis mix was added and incubated.

Quantitative polymerase chain reaction (qPCR) was conducted using ViPrimePLUS Taq qPCR Green Master Mix I (SYBR® Green Dye) (Vivantis, Malaysia). Each qPCR reaction included a cDNA template, forward and reverse primers, Taq qPCR Green Master Mix I, and water. Primers for ERα (ESR1), ERβ (ESR2), c-KIT (kit), and SCF (kitlg) and housekeeping genes B-actin and Cyclin A2 (Table 2) were

used, with primer efficiency established through dilution series and standard curve analysis. The qPCR assay protocol ran for 45 cycles, and a melt curve analysis was performed to confirm specific gene products. This analysis involved increasing the temperature in 5°C increments from 60 to 90°C.

The mRNA expression levels of each gene were determined using cycle threshold (Ct) values. A comparative method was employed, involving the normalization of the Ct values for the genes of interest (ER α , ER β , c-KIT, and SCF) with housekeeping genes (β -actin and Cyclin A2). This involved calculating Δ Ct by subtracting the average Ct value of housekeeping genes from the average Ct value of the gene of interest. Then, the $\Delta\Delta$ Ct for each sample was calculated by subtracting the Δ Ct of the Control group's average from the sample's Δ Ct. Finally, for each experimental sample, the gene expression fold-change was calculated and converted to log2. The log2 fold change was used to equalize the scales of up- and down-regulated genes. A positive log2 fold change indicates that the gene is upregulated, whereas a negative log2 fold change indicates that it is downregulated.

2.11 | Statistical analysis

Statistical analysis was conducted using SPSS version 26. One-way ANOVA was initially done, followed by a Tukey's HSD post hoc. The selection of the Tukey's HSD test was based on the study's equal sample sizes across groups, allowing for pairwise comparisons of group means. For data that did not follow a normal distribution (Kolmogorov-Smirnov: $p < 0.05$) curve, the Kruskal-Wallis test was employed. A significance level of $p < 0.05$ was used to determine statistical significance for both tests.

3 | RESULTS

3.1 | Organ coefficient

Wi-Fi exposure and EBN supplementation did not significantly affect reproductive organs (Figure 1). Conversely, E2 treatment led to a significant reduction in the organ coefficient of the seminal vesicle. This is evident in both the Control E2 and Wi-Fi+E2 groups, both of which displayed significantly lower seminal vesicle organ coefficients in comparison with the Control group ($p < 0.01$, < 0.01) and the Wi-Fi group ($p < 0.01$, 0.001).

3.2 | Sperm parameters

As illustrated in Figure 2, exposure to Wi-Fi significantly reduced sperm concentration ($p = 0.004$), motility ($p = 0.020$), and viability ($p = 0.002$). Meanwhile, supplementation with EBN reversed this decline, leading to a significant increase in sperm concentration in the Wi-Fi+EBN group ($p < 0.01$), surpassing the effect of E2 treatment ($p < 0.01$). Nonetheless, it could not restore sperm motility ($p = 0.400$) and viability ($p = 0.647$). Similarly, in a Wi-Fi environment, E2 treatment also reinstated normal sperm concentration ($p = 0.034$) but exhibited no significant impact on sperm motility ($p = 0.182$) and viability ($p = 0.997$).

While EBN and E2 showed similar effects in Wi-Fi-exposed environments, their effects differed in controlled environments. EBN supplementation significantly increased sperm concentration ($p < 0.01$) and sperm viability ($p = 0.003$) in a non-Wi-Fi environment, surpassing the effect of E2 treatment. E2 treatment had no significant effect on sperm concentrations ($p = 0.956$) and

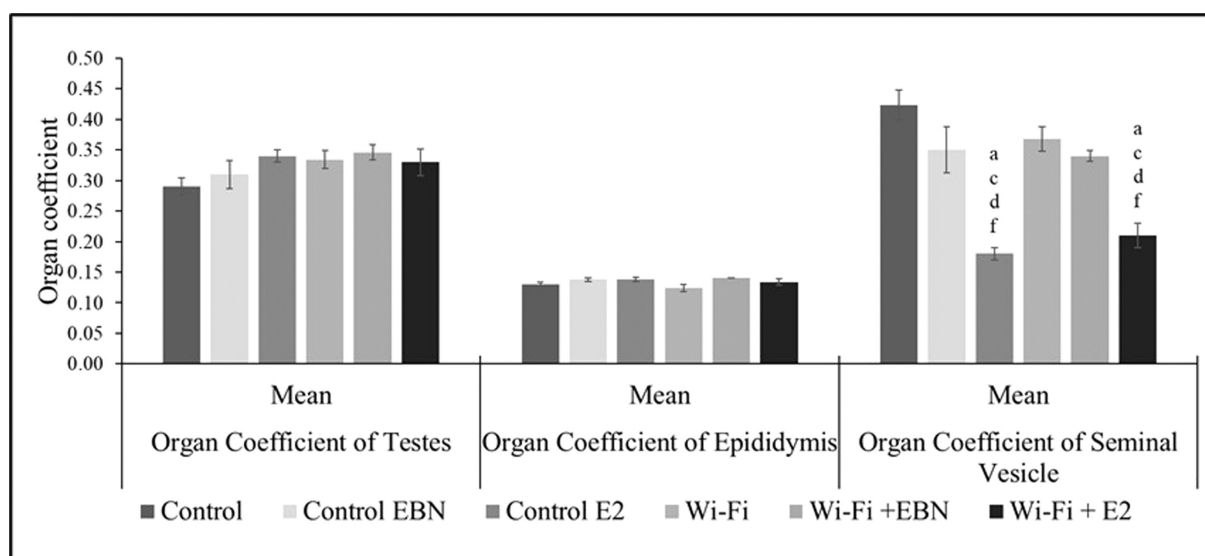


FIGURE 1 Organ coefficient of male reproductive organs for each group. Results are expressed as mean $\pm$ standard error of the mean (SEM) with $n = 6$ for each group. Significant results (p -value < 0.05) are indicated as ^asignificant compared to the Control group, ^csignificant compared to the Control EBN group, ^dsignificant compared to the Wi-Fi group, ^fsignificant compared to the Wi-Fi+EBN group.

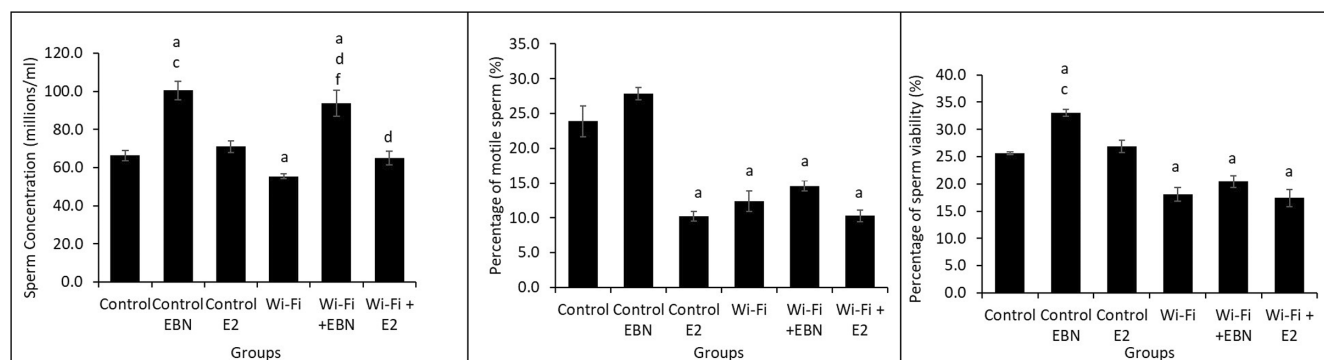


FIGURE 2 Sperm concentration, percentage of sperm motility and viability for each group. Results are expressed as mean $\pm$ standard error of the mean (SEM) with $n=6$ for each group. Significant results (p -value <0.05) are indicated as ^asignificant compared to the Control group, ^csignificant compared to the Control E2 group, ^dsignificant compared to the Wi-Fi group, ^fsignificant compared to Wi-Fi+E2 group.

viability ($p=0.970$), but it did lead to a substantial reduction in motility ($p=0.001$).

3.3 | Serum follicle-stimulating hormone, luteinizing hormone, testosterone, and estradiol levels

This study assessed the impact of Wi-Fi exposure and EBN supplementation on hormone levels by calculating the differences between post- and pretreatment serum hormone levels. Exposure to Wi-Fi resulted in reduced FSH ($p=0.014$) and testosterone levels ($p=0.001$), with LH ($p=0.431$) and E2 levels ($p=1.000$) remaining unaffected.

EBN supplementation in a Wi-Fi environment restored FSH ($p=0.022$) and testosterone to normal levels ($p=0.003$), increased LH levels ($p=0.003$), and decreased E2 levels ($p=0.011$). Interestingly, EBN elevated LH levels, outperforming the effect of E2 treatment ($p=0.004$). However, these effects of EBN supplementation were not observed in controlled environments, where it reduced serum FSH levels ($p=0.026$) but was still within the normal range. It also had no significant impact on LH ($p=1.000$), testosterone ($p=0.051$), and E2 levels ($p=1.000$).

On the other hand, E2 restored the suppressed FSH levels ($p=0.024$) and had no significant effect on LH levels ($p=1.000$). It also reduced testosterone levels ($p=0.019$) and increased E2 levels ($p=0.005$). Meanwhile, E2 treatment in controlled environments showed no significant effect on FSH levels ($p=0.937$), LH levels ($p=1.000$), E2 levels ($p=0.686$), but did lead to a decrease in testosterone levels ($p<0.01$). The comparison of the pretreatment, post-treatment and differences between post- and pretreatment serum hormone levels for all reproductive hormones in each group was summarized in Table 3.

3.4 | Testosterone/estradiol ratio (T/E2)

The Testosterone/Estradiol Ratio (T/E2) was calculated for each group. Lower T/E2 ratios were observed in both E2 treatment

groups, as illustrated in Figure 3. This decline was due to increased serum E2 levels and decreased serum testosterone levels.

Wi-Fi exposure did not significantly alter the T/E2 ratio ($p=0.285$). Meanwhile, Wi-Fi exposure combined with EBN supplementation significantly increased the T/E2 ratio compared to the Wi-Fi group ($p=0.029$) and the Wi-Fi+E2 group ($p=0.035$). This result demonstrates that EBN supplementation positively impacted the T/E ratio in Wi-Fi-exposed rats.

3.5 | Evaluation of estrogen receptors, c-KIT and stem cell factor mRNA expression by qPCR

Wi-Fi exposure reduced ER α mRNA expression ($p=0.037$) while increasing ER β mRNA expression significantly ($p=0.010$), as depicted in Figure 4. However, EBN supplementation in the Wi-Fi environment effectively normalized ER α mRNA expression in Wi-Fi-exposed rats ($p=0.014$), surpassing the effect observed in the Wi-Fi+E2 group ($p=0.011$). Additionally, EBN supplementation significantly increased ER β mRNA expression in Wi-Fi-exposed rats ($p<0.010$). Similar effects were also observed in the controlled environment, where both EBN supplementation and E2 treatment significantly increased ER α mRNA ($p=0.004$, 0.011) and ER β mRNA expression ($p=0.001$, <0.01).

Regarding c-KIT and SCF mRNA expression, Wi-Fi exposure increased c-KIT and SCF mRNA expression more than Control ($p<0.01$, 0.016). Similarly, Wi-Fi+EBN significantly increased c-KIT and SCF mRNA expression compared with Control ($p=0.031$, 0.036). Meanwhile, Wi-Fi+E2 only increased SCF mRNA expression compared with Control ($p<0.01$). Equally, in the controlled environment, both EBN supplementation and E2 treatment experienced significantly increased c-KIT ($p<0.01$, <0.01) and SCF mRNA expression ($p<0.01$, <0.01).

In summary, Wi-Fi exposure reduced ER α mRNA expression but increased ER β , c-KIT, and SCF mRNA expression. However, EBN and E2 supplementation had differing effects. EBN showed potential in normalizing ER α and enhancing c-KIT mRNA expression, whereas E2

TABLE 3 Pretreatment, post-treatment and differences in post- and pretreatment serum reproductive hormone levels in each group.

Group	FSH (ng/mL)			LH (mIU/mL)			T (ng/mL)			E2 (pg/mL)		
	Pretreatment	Post-treatment	Differences	Pretreatment	Post-treatment	Differences	Pretreatment	Post-treatment	Differences	Pretreatment	Post-treatment	Differences
Control	44.00 ± 3.0	58.63 ± 2.0	14.63 ± 1.8	3.48 ± 0.5	4.40 ± 0.5	1.20 ± 0.5	2.84 ± 0.4	3.49 ± 0.4	1.21 ± 0.6	7.38 ± 0.5	7.08 ± 0.6	-0.19 ± 0.4
Control EBN	47.17 ± 3.1	53.68 ± 4.0	6.51 ± 2.5 ^a	3.60 ± 0.3	4.15 ± 0.5	0.30 ± 0.2	3.14 ± 0.6	5.46 ± 0.6 ^{a,c}	2.61 ± 0.3	8.19 ± 0.7	8.15 ± 0.5	-0.30 ± 1.0
Control E2	36.86 ± 5.5	54.96 ± 4.9	19.12 ± 6.1	4.29 ± 0.3	6.03 ± 0.5 ^a	1.33 ± 0.7	4.39 ± 0.4	1.68 ± 0.2 ^a	-2.80 ± 0.1 ^a	7.77 ± 1.1	9.00 ± 1.2	1.06 ± 0.9
Wi-Fi	47.02 ± 2.9	46.92 ± 3.9 ^a	-2.06 ± 1.3 ^a	3.52 ± 0.5	3.58 ± 0.3	0.06 ± 0.4	4.78 ± 0.6	2.37 ± 0.2 ^a	-2.39 ± 0.7 ^a	9.14 ± 0.7	7.43 ± 0.2	-0.34 ± 0.6
Wi-Fi+EBN	44.50 ± 5.7	64.33 ± 4.3	14.48 ± 4.6 ^d	3.33 ± 0.4	6.29 ± 0.4 ^d	2.79 ± 0.3 ^{a,d,f}	2.90 ± 0.3	4.50 ± 0.6 ^d	1.60 ± 0.7 ^d	8.96 ± 0.6	5.70 ± 0.3 ^{a,d}	-3.25 ± 0.6 ^{a,d}
Wi-Fi+E2	46.63 ± 5.1	57.88 ± 5.7	8.50 ± 3.9 ^d	3.69 ± 0.7	4.58 ± 0.5	0.89 ± 0.4	4.45 ± 0.9	3.61 ± 0.6	-0.84 ± 0.6 ^a	6.57 ± 0.4	9.69 ± 1.0	3.12 ± 0.7 ^{a,d,e}

Note: Data are presented in mean ± standard error of the mean (SEM) with $n = 6$ for each group. Significant results (p -value < 0.05) are indicated as ^asignificant compared to the Control group, ^csignificant compared to the Control E2 group, ^dsignificant compared to the Wi-Fi group, ^esignificant compared with the Wi-Fi+EBN group, ^fsignificant compared to the Wi-Fi+E2 group.

treatment did not impact ER, c-KIT, and SCF mRNA expression in Wi-Fi-exposed rats.

3.6 | Evaluation of estrogen receptors, c-KIT and stem cell factor protein expression by Western blot

The expression of ER and ER proteins in western blot analysis indicated no statistically significant differences between experimental groups, ($p = 0.440, 0.086$) (Figure 5). c-KIT protein expression, similar to mRNA expression, demonstrated a substantial increase in the Control EBN group compared with the Control and Control E2 groups ($p = 0.005, 0.023$). However, c-KIT protein expression was not significantly different in the Wi-Fi, Wi-Fi+EBN, and Wi-Fi+E2 groups compared with the Control group ($p = 0.947, 0.901, 0.796$). Furthermore, the relative intensity of SCF protein expression was unaffected by Wi-Fi exposure, EBN supplementation, and E2 treatment in both Wi-Fi-exposed and nonexposed conditions. Overall, the results suggest that Wi-Fi exposure, EBN supplementation, and E2 treatment did not significantly influence the expression of ER α , ER β , c-KIT, and SCF proteins in the testes. The exception was observed in the Control EBN group, where c-KIT protein expression was significantly increased compared to the Control and Control E2 groups.

4 | DISCUSSION

This study explored the potential benefits of supplementing with EBN to protect male reproductive health from the impacts of Wi-Fi radiation. We found that Wi-Fi exposure decreased sperm quality, including concentration, motility, and viability. The discussion focused on the mechanisms behind these effects and how EBN supplementation could counteract them.

4.1 | Effect of Wi-Fi on male reproductive health

Wi-Fi exposure has been shown to affect male reproductive health through multiple pathways, involving both primary and secondary testicular dysfunction. Secondary testicular dysfunction arises as Wi-Fi exposure also affects the hypothalamic-pituitary-gonadal (HPG) axis, leading to hormonal imbalances that indirectly impair testicular function. In our study, exposure to 2.45 GHz Wi-Fi decreased FSH and testosterone levels, while LH and E2 levels showed no significant changes. The unchanged LH levels could be attributed to feedback mechanisms compensating for the decrease in testosterone. The reduction in FSH and testosterone levels is believed to influence spermatogenesis, resulting in decreased sperm production and concentration. Similarly, previous studies showed RF-EMF exposure had reduced LH, FSH, and testosterone, resulting in low sperm count.^{15,16}

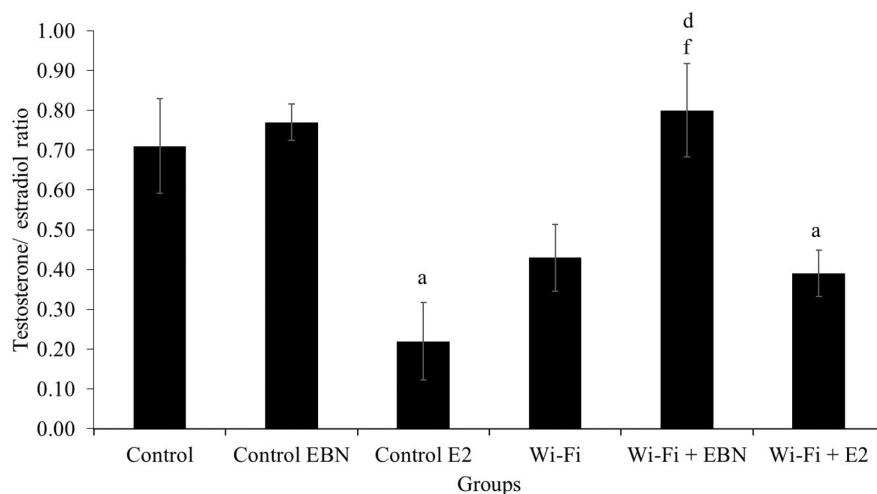


FIGURE 3 T/E2 ratio for each group. Results are expressed as mean $\pm$ standard error of the mean (SEM) with $n=6$ for each group. Significant results (p -value <0.05) are indicated as ^asignificant compared to the Control group, ^dsignificant compared with the Wi-Fi group, ^fsignificant compared to Wi-Fi+E2 group.

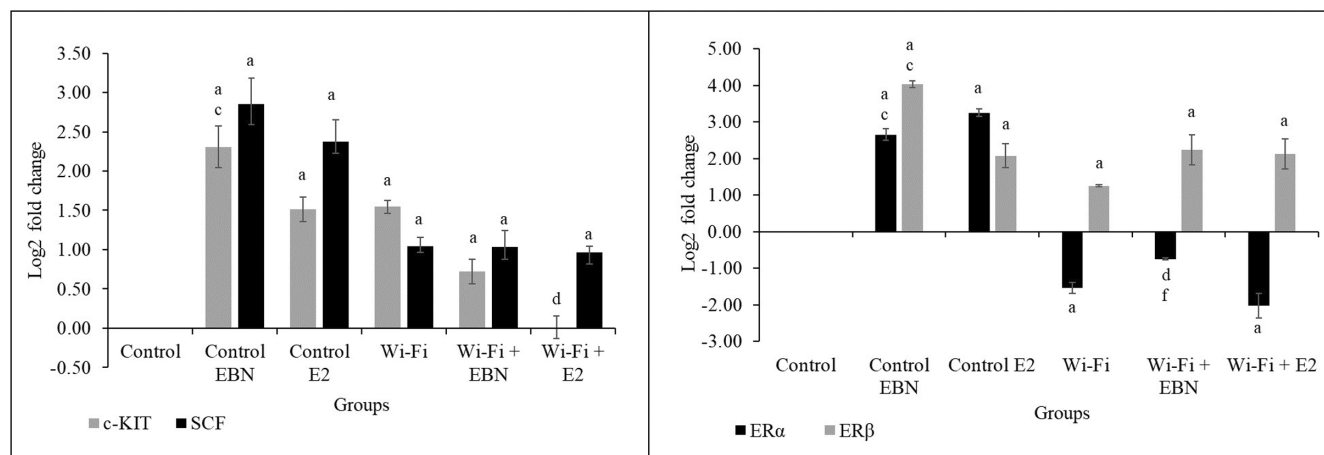


FIGURE 4 Log2 fold change of ER α , ER β , c-KIT and SCF mRNA expression in each group. After normalization with β -actin and cyclin A2 housekeeping genes, mRNA expression was determined by qPCR. Data are expressed as fold change variation relative to the Control group (0 baselines) and presented as mean $\pm$ SEM ($n=6$). Significant results (p -value <0.05) are indicated as ^asignificant compared to the Control group, ^csignificant compared to the Control E2 group, ^dsignificant compared to the Wi-Fi group, ^fsignificant compared to the Wi-Fi+E2 group.

The impact of RF-EMF on the HPG axis involves both thermal and nonthermal effects. A simulation study assessed the thermal influence of RF-EMF at various frequencies on the hypothalamus and pituitary gland. It found that RF-EMF raised organ temperature, with the highest increase observed at 900 and 2100 MHz.¹⁷ This suggests a potential thermal effect of Wi-Fi RF-EMF on specific brain regions.¹⁷

Additionally, Wi-Fi RF-EMFs may induce a nonthermal effect by triggering oxidative stress in the hypothalamus and pituitary gland. Exposure to dual-band EGSM 900/1800 MHz RF-EMF led to elevated levels of malondialdehyde (MDA), a marker of oxidative stress, and decreased antioxidant enzyme superoxide dismutase. This resulted in increased oxidative stress.¹⁶ Furthermore, exposure to 1900 MHz RF-EMF increased oxidative stress and

inflammation in the hippocampus.¹⁸ These effects may disrupt the HPG axis and influence gonadotropin secretion. However, the precise mechanism of RF-EMF's impact on the HPG axis requires further investigation.

Despite normal LH levels, the observed decline in testosterone levels could be attributed to RF-EMF impairing Leydig cell function, reducing testosterone production. This aligns with previous research by Saygin et al.¹⁹ showing decreased testosterone, Leydig, and Sertoli cell degeneration and sperm concentration after 2.45 GHz EMF exposure. This damage to Leydig cells is likely caused by increased oxidative stress, disrupting testosterone synthesis.²⁰ RF-EMF exposure may also lead to LH receptor dysfunction in Leydig cells²¹ and Leydig cell apoptosis.²² Henceforth, this had resulted in lowered testosterone levels.

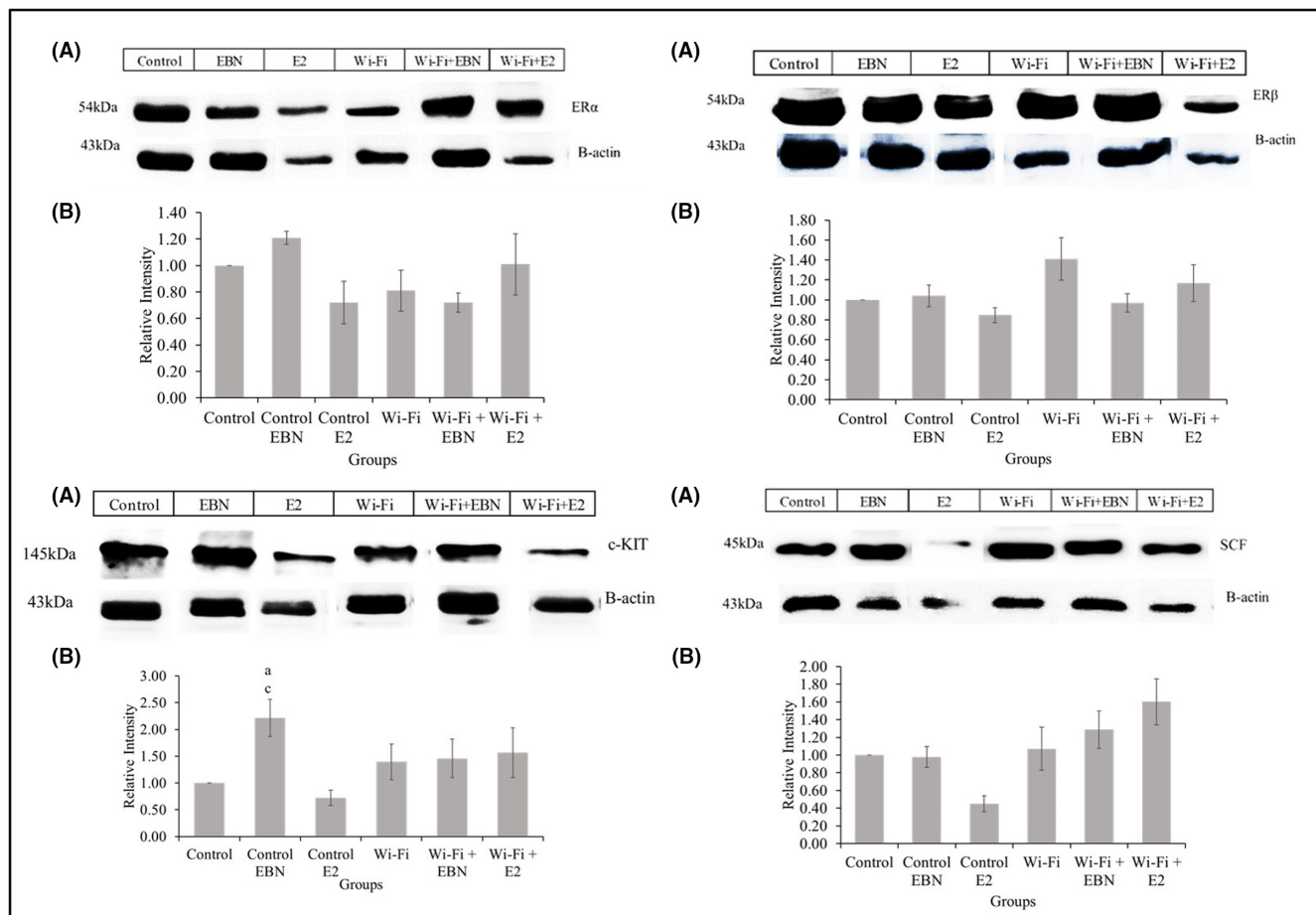


FIGURE 5 Protein expression of c-KIT in the testes. (A) The c-kit and β -actin protein bands on western blots for six experimental groups. (B) The relative intensity of c-kit protein expression after normalization with β -actin. Results are expressed as mean $\pm$ standard error of the mean (SEM) with $n=6$ for each group. Significant results (p -value <0.05) are indicated as ^asignificant compared to the Control group, ^csignificant compared with the Control E2 group.

Moreover, this study discovered that Wi-Fi exposure had no effect on serum E2 levels. The testes produce approximately 20%–50% of circulating E2 in males, with the remainder produced through the peripheral aromatization of testosterone in adipose tissue, brain, muscle, and bone.⁸ According to one study, 2100MHz exposure elevated serum aromatase levels in rats.¹⁵ This observation of RF-EMF-induced aromatization shows that, despite a decrease in serum testosterone levels, the study's normal serum E2 level could be attributed to increased aromatization activity in numerous organs.

In addition to its impact on hormones in the HPG axis, RF-EMF can also directly affect the testes, through primary testicular dysfunction. Primary testicular dysfunction occurs as RF-EMF from Wi-Fi can directly impact the testes, causing significant harm to the seminiferous tubules and Sertoli cells, which are critical for spermatogenesis. This study discovered that eight weeks of 2.45GHz Wi-Fi exposure had reduced sperm concentration, motility, and viability in rats. The reduction in sperm quality is consistent with the findings of Shokri et al.,⁷ who discovered a decrease in sperm concentration, motility, and normal morphology after exposure to 2.45 GHz Wi-Fi. According to a comprehensive review of animal and human studies on the effect of RF-EMF radiation on sperm quality,

RF-EMF had the most significant impact on sperm concentration, morphology, and motility.⁵

The direct impact mechanism of RF-EMF on the testes may be attributed to its thermal effect, as the testes are known to be particularly sensitive to thermal stress. Maintaining temperatures lower than the body's core is essential for the optimum spermatogenesis.²³ RF-EMF exposure, especially near the testes, can raise local temperatures, causing testicular hyperthermia that disrupts spermatogenesis.²⁴

Additionally, RF-EMF exposure induces oxidative stress, leading to increased production of reactive oxygen species (ROS).²⁵ This oxidative stress damages testicular cells and sperm, reducing sperm viability, motility, and DNA integrity. Other than that, nonthermal effects of RF-EMF involve disruptions in sperm signal transduction involving calcium homeostasis. Calcium ions (Ca^{2+}) are critical in sperm function, including motility and fertilization. RF-EMF exposure disrupts calcium balance, reducing Ca^{2+} levels and impairing sperm motility, contributing to the decline in sperm quality.²⁴

In this study, we also investigated testicular estrogenic activity by examining the expression of estrogen receptors (ER α and ER β). ER α

is the functionally dominant ER that regulates spermatogenesis and maintains sperm morphology and motility.²⁶ Besides, ER α disruption decreased the number of germ cells in the testes.⁸ Meanwhile, ER β is more widely spread in the testes, including Leydig and Sertoli cells, seminiferous tubules, spermatogonia, and spermatocytes, with the highest levels in Sertoli cells.²⁷ ER β plays a minor role in male reproduction, primarily in spermiation, the release of mature spermatids into the seminiferous tubule before their journey to the epididymis.

According to this study, Wi-Fi exposure alters estrogen receptor (ER) expression in the testes, reducing ER α mRNA while increasing ER β mRNA. However, these changes were not reflected in protein expression. Although protein levels showed no significant difference, there is a similar trend of decreased ER α and increased ER β in Wi-Fi-exposed rats. We hypothesize that the decrease in ER α mRNA expression is caused by RF-EMF-induced Leydig cell destruction. As ER α is abundantly found in Leydig cells,²⁸ reducing their cell number may lead to less expression ER α detection.

The increase in ER β mRNA may be linked to RF-EMF-triggered shifts in sperm cell cycle progression.²⁷ Research has demonstrated that RF-EMF exposure can lead to spermatogonia arrest, primarily attributed to oxidative stress. This affects germ cell populations, increasing undifferentiated spermatogonia, a cell type known to predominantly express ER β mRNA.²⁷ This could explain the rise in ER β mRNA expression seen in our study. Nonetheless, the exact effects of RF-EMF on ER expression in the testes and the underlying mechanisms warrant further investigation. In summary, this study showed that Wi-Fi interfered with male reproductive hormone production in the HPG axis and testicular function in spermatogenesis. Consequently, it decreased sperm quality and male infertility.

In addition to our findings, future studies should address several key areas. These include examining the effects of prolonged Wi-Fi exposure on male fertility and investigating underlying molecular mechanisms such as p38 and MAPK14, which play roles in cellular stress responses.²⁹ Additionally, research should assess the impact of different RF-EMF frequencies and intensities on reproductive health, specifically comparing the effects of 2.45 and 5 GHz Wi-Fi on male reproductive function. While our study showed adverse impacts with 2.45 GHz Wi-Fi, 5 GHz Wi-Fi may have different biological effects. Evaluating thermal and nonthermal mechanisms, such as oxidative stress markers and tissue temperature changes, will help determine whether 5 GHz Wi-Fi poses a lesser risk, leading to better Wi-Fi exposure guidelines. Furthermore, validating these findings in human through epidemiological studies and clinical trials will be essential for translating our observations into clinical practice and public health guidelines.

4.2 | The protective effect of EBN on male reproductive health in a Wi-Fi environment

The study also explored the potential protective effects of EBN, a supplement known for its beneficial properties that could benefit reproductive health. The supplementation spanned 8 weeks,

considering that rat spermatogenesis takes approximately 52 days,³⁰ thereby accommodating a cycle of new spermatogenesis. EBN supplementation effectively mitigated the adverse impacts of Wi-Fi radiation on male reproductive health. It restored suppressed FSH and testosterone levels and increased serum LH and sperm concentration.

Normalizing FSH and testosterone levels with EBN supplementation demonstrates its protective effect on the hypothalamic-pituitary-gonadal (HPG) axis. EBN's antioxidant, proliferative, and hormonal properties are believed to protect the hypothalamus, pituitary, and gonadal cells significantly. EBN contains antioxidants such as amino acids, vitamins, and lactoferrin, which help reduce oxidative stress and protect cells from damage.³¹ In rats, EBN supplementation significantly increased serum enzymatic antioxidants and total antioxidant status, demonstrating its potent antioxidant properties. It subsequently reduced oxidative stress in the rat uterus and preserved uterine histomorphology.³² This shows that EBN's antioxidant qualities may protect cells in the hypothalamus, pituitary gland, and testes from ROS generated by RF-EMF Wi-Fi, lowering oxidative stress.

Additionally, past study had reported that EBN's had proliferative properties—mainly through epidermal growth factor (EGF).³³ The ability to induce cell growth could have protected the hypothalamus, pituitary and testicular cells, thus minimized cell death and enhanced cell replacement. EGF binding to its receptor stimulates cell growth and hormone secretion in the pituitary.³⁴ Other previous studies have also shown similar characteristics. EBN had increased EGF expression, leading to a proliferation of gonadotrophic cells in the pituitary and enhanced secretion of hormones such as growth hormone and prolactin.³⁵ This implies that EBN's proliferative properties might protect the pituitary gland and testes, contributing to the restoration of serum FSH, testosterone, and elevated serum LH levels observed in this study.

EBN also contains male reproductive hormones such as testosterone, E2, FSH, and LH.² According to an Orbitrap LCMS analysis, the EBN extract used in this study had the highest testosterone concentration. Two other reproductive hormones, E2 and progesterone, were also mentioned.¹¹ These hormonal components of EBN may have contributed to increased serum FSH, LH, and testosterone levels observed in Wi-Fi-exposed rats supplemented with EBN.

A distinct feature of EBN is its estrogenic properties. In female rats, EBN notably increased plasma E2 and progesterone levels.¹ However, in our male rat study, EBN showed no significant effect on E2 levels under normal conditions. Yet, in Wi-Fi-exposed rats, EBN supplementation reduced serum E2 levels.

The decrease in E2 levels may be linked to the estrogenic-like properties of EBN in various tissues of male rats, leading to alterations in aromatase enzyme activity. The E2-like components of EBN could impact diverse tissues, such as the testes, adipose tissue, brain, muscle, and bone, causing them to interpret it as E2 and resulting in reduced aromatization. Consequently, this led to a decrease in the circulating E2 levels observed in our study. Supporting this idea, our study discovered normal or increased estrogenic activity in the

testes of EBN-supplemented groups, confirming the estrogenic impact of EBN in male rats.

Furthermore, the reduced aromatization increased testosterone levels, elevating the testosterone/E2 (T/E2) ratio in Wi-Fi-exposed rats supplemented with EBN. This ratio significantly influences sperm parameters, with a higher T/E2 ratio associated with better sperm quality. Correcting T/E2 ratios with treatments such as selective estrogen receptor modulators (SERMs) or aromatase inhibitors has been applied in male infertility cases.³⁶ Research utilizing aromatase inhibitors in males with low T/E2 ratios demonstrated significant post-treatment increases in T/E2 ratios, enhanced sperm concentration, and total motility count.³⁷ It is speculated that EBN supplementation, due to its estrogenic activity, functions similarly by augmenting the T/E2 ratio, contributing to an increased sperm concentration.

To better understand EBN's estrogenic activity and its potential to protect against Wi-Fi-induced damage, we examined the expression of ER in the testes. EBN supplementation enhanced ER expression, particularly ER α and ER β mRNA, reinforcing its estrogenic potency. Indeed, these changes were not mirrored in protein expression.

The connection between mRNA and protein expression displayed interesting patterns. Notably, alterations in receptor expression at the genetic level (mRNA) did not consistently align with changes at the protein level.³⁸ The reason may stem from a disruption in translational mechanism regulation, specifically ER α -dependent translational offsetting, influencing the translation of genes into proteins. This phenomenon maintains constant protein levels despite changes in mRNA abundance.³⁸

The impact of EBN's estrogenic activity on the testes remains uncertain, as previous research has primarily focused on female rats. In those studies, EBN consumption increased ER protein expression in the uterus, although the specific type of ER was not specified. Additionally, EBN intake increased plasma antioxidant levels and decreased oxidative stress, indicating its potential protective role.³⁵ Given this background, the observed enhancement in both ER α and ER β mRNA expression following EBN supplementation in Wi-Fi-exposed rats can likely be attributed to its combined estrogenic and antioxidant properties, which positively influence testicular function.

In summary, EBN's influence on estrogen receptors helps regulate estrogenic activity, leading to a higher T/E2 ratio and reducing estrogen's impact on testicular function. This effect could be due to aromatase inhibition, which supports increased testosterone production. Additionally, EBN enhances testosterone synthesis through its anti-inflammatory and antioxidant properties. Its anti-inflammatory effects reduce inflammation caused by Wi-Fi exposure, which can impair Leydig cell function essential for testosterone production.³⁹ The antioxidant properties of EBN also combat oxidative stress exacerbated by Wi-Fi exposure, which can damage Leydig cells.⁴⁰ This reduction in inflammation and oxidative damage supports healthier Leydig cell function and promotes testosterone synthesis, thereby supporting spermatogenesis and increasing sperm concentration.

This study also uncovered intriguing differences between EBN's estrogenic activity and E2 treatment. In Wi-Fi-exposed rats, E2 treatment significantly increased E2 levels, reducing testosterone levels. However, it restores suppressed FSH levels due to Wi-Fi exposure but does not affect LH levels. This reduction in testosterone production in both E2 treatment groups occurs because exogenous E2 inhibits the effect of LH on Leydig cells during steroidogenesis, affecting testosterone levels.⁹

The decrease in testosterone was also reflected in the organ coefficient of the seminal vesicle for E2 treatment groups, which were found to be reduced. A decrease in seminal vesicle weight is a highly sensitive indicator of low testosterone or antiandrogenic activity.⁴¹ This aligns with the observed decline in testosterone levels, highlighting the impact of E2 treatment on androgen-related physiological parameters. The changes in seminal vesicle weight suggest potential antiandrogenic effects linked to hormonal balance alterations induced by E2 treatment. Further investigation is needed to understand the specific mechanisms behind these observations.

The protective mechanism through which E2 treatment restores FSH levels in RF-EMF-exposed rats remains unclear. It is proposed that E2 shields the hypothalamus and pituitary gland from RF-EMF damage by reducing oxidative stress and inflammation. Notably, despite the decline in testosterone, E2 treatment in Wi-Fi-exposed environments restores sperm concentration. This might be because FSH's action can compensate for the missing testosterone stimulus, and a low intratesticular testosterone concentration is adequate for spermatogenesis.⁴²

E2 treatment normalizes serum FSH levels, whereas EBN supplementation, known for its antioxidant characteristics, not only normalizes FSH levels but also increases LH levels. This unique effect might be linked to EBN's proliferative impact, enhancing gonadotrophic cell numbers and their secretory functions.

Additionally, the study explored the c-KIT/SCF system. Wi-Fi exposure increased c-KIT and SCF mRNA expression, potentially associated with alterations in spermatogonia proliferation. EBN supplementation and E2 treatment affected c-KIT and SCF expression in controlled settings, emphasizing their potential role in spermatogonia proliferation. However, these effects were less pronounced in Wi-Fi-exposed groups.

The study also revealed that Wi-Fi exposure could disrupt male reproductive hormones, compromising sperm quality and fertility. EBN supplementation emerged as a promising strategy to mitigate these effects by restoring hormone levels, influencing ER expression, and potentially affecting the c-KIT/SCF system. The intricate relationship between mRNA and protein expression and complex mechanisms underscores the challenges in establishing a direct correlation.

5 | CONCLUSION

This study highlights the negative impact of eight weeks of Wi-Fi exposure on sperm quality, including decreased concentration, motility,

and viability, which can be ascribed to changes in male reproductive hormones. EBN supplementation appears to be a preventive intervention, significantly increasing gonadotrophin and testosterone levels, as well as sperm concentration in Wi-Fi-exposed rats, but not influencing sperm motility or viability. Furthermore, it increases the T/E2 ratio and restores estrogenic activity in the testes, resulting in enhanced sperm concentration. The study also reveals a discrepancy between the impacts of EBN supplementation and E2 treatment on sperm concentration in a Wi-Fi-exposed setting, emphasizing EBN's unique protective properties without adverse effects on male reproduction. In conclusion, EBN supplementation effectively restores spermatogenesis capabilities that are affected by Wi-Fi-induced damage. This is achieved through the modulation of male reproductive hormones, with a primary influence on sperm concentration. Nevertheless, further research is necessary to fully understand the mechanisms and establish safe usage limits for maximizing the benefits of EBN while minimizing potential risks.

ACKNOWLEDGMENTS

We gratefully acknowledge financial support from the Faculty of Medicine, UKM (Grant No. FF-2021-200) Special thanks to Glycofood Sdn Bhd for providing the EBN extract. We also appreciate the dedication of our laboratory personnel in animal care and technical support during the molecular study.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

ETHICS STATEMENT

This study was reviewed and approved by the Animal Ethics Committee UKM (UKMAEC) with approval number FISIO/FP/2021/SITI FATIMAH/24-MAR./1155-MAR.-2021-JULY-2022.

ANIMAL STUDIES

All procedures performed in studies involving animals were in accordance with the ethical standards of the Animal Ethics Committee UKM (UKMAEC).

ORCID

Sofwatul Mokhtarah Maluin  <https://orcid.org/0000-0002-5552-4632>

REFERENCES

- Albishtue A, Yimer N, Zakaria Z, Haron AW, Almhanawi B. Ameliorating effect of edible bird's nest against lead acetate toxicity on the rat hypothalamic-pituitary-ovarian axis and expressions of epidermal growth factor and vascular endothelial growth factor in ovaries. *Comp Clin Pathol*. 2018;27:1257–67.
- Ma F, Liu D. Extraction and determination of hormones in the edible bird's nest. *Asian J Chem*. 2012;24:117–20.
- Jaffar FHF, Osman K, Hui CK, Zulkefli AF, Ibrahim SF. Long-term Wi-Fi exposure from pre-pubertal to adult age on the spermatogonia proliferation and protective effects of edible bird's nest supplementation. *Front Physiol*. 2022;13:828578.
- Albishtue AA, Yimer N, Zakaria MZA, Haron AW, Babji AS, Abubakar AA, et al. Effects of EBN on embryo implantation, plasma concentrations of reproductive hormones, and uterine expressions of genes of PCNA, steroids, growth factors and their receptors in rats. *Theriogenology*. 2019;126:310–9.
- Kesari KK, Agarwal A, Henkel R. Radiations and male fertility. *Reprod Biol Endocrinol*. 2018;16(1):118.
- Jaffar FHF, Osman K, Ismail NH, Chin KY, Ibrahim SF. Adverse effects of Wi-Fi radiation on male reproductive system: a systematic review. *Tohoku J Exp Med*. 2019;248(3):169–79.
- Shokri S, Soltani A, Kazemi M, Sardari D, Babapour MF. Effects of Wi-Fi (2.45 GHz) exposure on apoptosis, sperm parameters and testicular histomorphometry in rats: a time course study. *Cell J*. 2015;17(2):322–31.
- Cooke PS, Nanjappa MK, Ko C, Prins GS, Hess RA. Estrogens in male physiology. *Physiol Rev*. 2017;97(3):995–1043.
- Schulster M, Bernie AM, Ramasamy R. The role of estradiol in male reproductive function. *Asian J Androl*. 2016;18(3):435–40.
- Carreau S, Bois C, Zanatta L, Silva FRMB, Bouraima-Lelong H, Delalande C. Estrogen signaling in testicular cells. *Life Sci*. 2011;89(15):584–7.
- Jaffar FHF, Osman K, Hui CK, Zulkefli AF, Ibrahim SF. Edible bird's nest supplementation improves male reproductive parameters of Sprague Dawley rat. *Front Pharmacol*. 2021;12:631402.
- Yida Z, Imam MU, Ismail M, Hou Z, Abdullah MA, Ideris A, et al. Edible bird's nest attenuates high fat diet-induced oxidative stress and inflammation via regulation of hepatic antioxidant and inflammatory genes. *BMC Complement Altern Med*. 2015;15:310.
- Olfati A, Moghaddam G, Baradaran B. FSH and estradiol benzoate administration recover spermatogenesis and sexual hormone levels in a busulfan-injured rat model. *Comp Clin Pathol*. 2020;29(1):53–9.
- Zhang R, Zhang L, Jiang D, Zheng K, Cui Y, Li M, et al. Mouse organ coefficient and abnormal sperm rate analysis with exposure to tap water and source water in Nanjing reach of Yangtze River. *Ecotoxicology*. 2014;23(4):641–6.
- Khoshbakht S, Motejaded F, Karimi S, Jalilvand N, Ebrahimzadeh-Bideskan A. Protective effects of selenium on electromagnetic field-induced apoptosis, aromatase P450 activity, and leptin receptor expression in rat testis. *Iran J Basic Med Sci*. 2021;24(3):322–30.
- Oyewopo AO, Olaniyi SK, Oyewopo CI, Jimoh AT. Radiofrequency electromagnetic radiation from cell phone causes defective testicular function in male Wistar rats. *Andrologia*. 2017;49(10):e12772.
- Uluaydin K, Cerezci O, Şeker S. Can mobile phone usage affect hypothalamus-pituitary-adrenal axis response? 2020 10th Annual Computing and Communication Workshop and Conference (CCWC); 2020. p. 780–3.
- Singh KV, Gautam R, Meena R, Nirala JP, Jha SK, Rajamani P. Effect of mobile phone radiation on oxidative stress, inflammatory response, and contextual fear memory in Wistar rat. *Environ Sci Pollut Res Int*. 2020;27(16):19340–51.
- Saygin M, Asci H, Ozmen O, Cankara FN, Dincoglu D, Ilhan I. Impact of 2.45 GHz microwave radiation on the testicular inflammatory pathway biomarkers in young rats: the role of gallic acid. *Environ Toxicol*. 2016;31(12):1771–84.
- Wang H, Zhang X. Magnetic fields and reactive oxygen species. *Int J Mol Sci*. 2017;18(10):2175.
- Sepehrmanesh M, Saeb M, Nazifi S, Kazemipour N, Jelodar G, Saeb S. Impact of 900 MHz electromagnetic field exposure on main male reproductive hormone levels: a *Rattus norvegicus* model. *Int J Biometeorol*. 2014;58(7):1657–63.
- Saygin M, Caliskan S, Karahan N, Koyu A, Gumral N, Uguz A. Testicular apoptosis and histopathological changes induced by a 2.45 GHz electromagnetic field. *Toxicol Ind Health*. 2011;27(5):455–63.

23. Durairajanayagam D, Agarwal A, Ong C. Causes, effects and molecular mechanisms of testicular heat stress. *Reprod Biomed Online*. 2015;30(1):14–27.
24. Agarwal A, Singh A, Hamada A, Kesari K. Cell phones and male infertility: a review of recent innovations in technology and consequences. *Int Braz J Urol*. 2011;37(4):432–54.
25. Kamali K, Taravati A, Sayyadi S, Gharib FZ, Maftoon H. Evidence of oxidative stress after continuous exposure to Wi-fi radiation in rat model. *Environ Sci Pollut Res Int*. 2018;25(35):35396–403.
26. Joseph A, Shur B, Ko C, Chambon P, Hess R. Epididymal hypo-osmolality induces abnormal sperm morphology and function in the estrogen receptor alpha knockout mouse. *Biol Reprod*. 2010;82:958–67.
27. Pandey N, Giri S, Das S, Upadhaya P. Radiofrequency radiation (900 MHz)-induced DNA damage and cell cycle arrest in testicular germ cells in swiss albino mice. *Toxicol Ind Health*. 2017;33(4):373–84.
28. Dostalova P, Zatecka E, Dvorakova-Hortova K. Of oestrogens and sperm: a review of the roles of oestrogens and estrogen receptors in male reproduction. *Int J Mol Sci*. 2017;18(5):904.
29. Azhar NA, Osman K, Jaffar FHF, Chua KH, Mat Ros MF, Zulkefli AF, et al. Effect of edible bird nest supplementation against busulfan-induced oligospermia in adult rats. *Sains Malays*. 2024;53:1219–34.
30. Cordeiro DA Jr, Costa GMJ, França LR. Testis structure, duration of spermatogenesis and daily sperm production in four wild cricetid rodent species (*A. cursor*, *A. montensis*, *N. lasiurus*, and *O. nigripes*). *PLoS ONE*. 2021;16(5):e0251256.
31. Ghassem M, Arihara K, Mohammadi S, Sani NA, Babji AS. Identification of two novel antioxidant peptides from edible bird's nest (*Aerodramus fuciphagus*) protein hydrolysates. *Food Funct*. 2017;8(5):2046–52.
32. Quddus A, Yimer N, Jesse FFA, Basit MA, Amir M, Islam MS. Edible bird's nest protects histomorphology of rat's uterus against cadmium (Cd) toxicity through a reduction of Cd deposition and enhanced antioxidant activity. *Saudi J Biol Sci*. 2021;28(12):7068–76.
33. Bai W, Deng F, Zhang X, Han Y, Ye X, Wang N, et al. The determination of epidermal growth factor in edible bird's nest by enzyme-linked immunosorbent assay. *Appl Biol Chem*. 2023;66:1–9.
34. Chen J, Zeng F, Forrester SJ, Eguchi S, Zhang MZ, Harris RC. Expression and function of the epidermal growth factor receptor in physiology and disease. *Physiol Rev*. 2016;96(3):1025–69.
35. Albishtue AA, Yimer N, Zakaria MZA, Haron AW, Yusoff R, Assi MA, et al. Edible bird's nest impact on rats' uterine histomorphology, expressions of genes of growth factors and proliferating cell nuclear antigen, and oxidative stress level. *Vet World*. 2018;11(1):71–9.
36. Rambhatla A, Mills JN, Rajfer J. The role of estrogen modulators in male hypogonadism and infertility. *Rev Urol*. 2016;18(2):66–72.
37. Abhyankar N, Shoshany O, Niederberger C. Testosterone to estradiol ratio correlates with sperm concentration improvement in hypogonadal oligozoospermic patients treated with anastrozole. *Fertil Steril*. 2016;106:e239–e240.
38. Lorent J, Kusnadi EP, van Hoef V, Rebello RJ, Leibovitch M, Ristau J, et al. Translational offsetting as a mode of estrogen receptor α -dependent regulation of gene expression. *EMBO J*. 2019;38(23):e101323.
39. Lee CH, Hamdan N, Nyakuma BB, Wong SL, Wong KY, Jamaluddin H, et al. Functional and biological activities of edible bird's nest (EBN) protein by proteomic and bioinformatic analyses. *J Food Meas Charact*. 2024;18(4):3018–31.
40. Monageng E, Offor U, Takalani NB, Mohlala K, Opuwari CS. A review on the impact of oxidative stress and medicinal plants on Leydig cells. *Antioxidants*. 2023;12(8):1559.
41. OECD 2010 : Oecd. 2010. Histopathological Evaluation. <https://www.oecd.org/chemicalsafety/testing> [25 May 2023].
42. Oduwole OO, Peltoketo H, Huhtaniemi IT. Role of follicle-stimulating hormone in spermatogenesis. *Front Endocrinol (Lausanne)*. 2018;9:763.

How to cite this article: Maluin SM, Jaffar FHF, Osman K, Zulkefli AF, Mat Ros MF, Ibrahim SF. Exploring edible bird nest's potential in mitigating Wi-Fi's impact on male reproductive health. *Reprod Med Biol*. 2024;23:e12606. <https://doi.org/10.1002/rmb2.12606>